

Module 9a: Other Compliance/Noncompliance: Salmonella Sampling

Sampling Raw Meat and Poultry for *Salmonella* Analysis

As part of the Food Safety and Inspection Service's Pathogen Reduction/HACCP Rule, performance standards were set for levels of *Salmonella* on raw meat and poultry. All plants that slaughter swine, beef, chickens and turkeys must ensure that their *Salmonella* contamination rate is below the current national baseline incidence. The same is true for plants that grind beef, pork, chicken or turkey. We expect contamination to be on raw product. But we do not expect to encounter high numbers of pathogenic bacteria. Pathogens are organisms capable of causing human illness.

It is important to have good aseptic sampling techniques and follow the step-by-step procedure when sampling these products.

This video highlights portions of the [Salmonella Analysis](#) guidebook and illustrates all the steps in sample collection for swine, beef, chicken, turkey and ground product. First, we'll show you what supplies will be needed, then how to select your sample, prepare for sample collection using the aseptic technique, actually take a sample and finally how to store and ship samples.

Let's look at what supplies you can expect to receive from the **Food Hazard Surveillance** in Washington, D.C. You should get preprinted FSIS Forms 10,210-7. These will be individually numbered. ~~Also, there will be a master list, like this one, of all the sampling dates for the upcoming month. You can use this for tracking the samples you collect. However, this is not an official form that you must use, but merely a quick-glance list for your benefit.~~ You also should receive bar code stickers that you must use on each sample bag or container that identifies the type of sample.

In the event samples are not collected for one reason or another, you'll also receive pre-addressed laboratory mailing labels. Use these labels to send **one** completed sample request form **along with the entire set of sample requests forms to the laboratory designated on the label.** Check the appropriate box in the **"Reason if Uncollected" section.**

The pre-addressed FedEx Billable Stamp Receipts for sample overnight delivery to the designated laboratory and the actual sampling supplies will come to you from the FSIS ~~Technical Service~~ Laboratories. These include the shipping container, sterile gloves, and gel-packs, and depending upon the type of sample requested, a sponge, buffered peptone water, a template, a zip-lock type bag, a screw-cap jar, a Whirl-Pak® bag, and a sterile plastic ring wrapped in a sterile plastic sheet. The shipping containers are color coded for each type of product: neon orange for beef and swine, neon green for poultry, and neon pink for ground product.

The day or so before you collect the sample, prepare your supplies. Check inside the shipping container to make sure everything is there for the type of sample you will be taking. For all carcass samples, you will need buffered peptone water. Examine this carefully. If it is cloudy or turbid or has particulate matter in it, do not use. Instead, clearly mark it "Bad" and send it back to the supplying lab. If it is clear, place it in a

secure refrigerator. Put the gel-packs in a freezer and the open shipping container in either a cooler or refrigerator to prechill.

Before selecting the sample, you need to determine the sampling location. This location must be safe and accessible. For beef or swine, it should be in a cooler and can be at a transfer chain, a rail, or the like. These carcasses should have been chilled for at least 12 hours. Poultry carcasses will be selected after chilling, at the end of the drip line or the last accessible point prior to packing or cut-up. Livestock or poultry carcasses to be hot-boned may be sampled after the final wash and prior to hot-boning.

Ground product must obviously be sampled after grinding, and if possible, before any spices or seasoning is added. It must also be sampled prior to final packaging.

All sample units have to be randomly selected. That means each sample unit has an equal chance of being chosen. A sample unit is a half-carcass for beef and a whole carcass for swine and poultry. Ground product is the 25-grams that you select. Once the sample location has been selected, randomly determine at what time you will actually take the sample. If more than one chiller or grinder are in operation, randomly select from which chiller or grinder to take the sample. The same applies for chilled carcasses in more than one cooler or on more than one chain or rail.

When you go out to collect the sample from the selected location at the randomly determined time, don't choose the carcass that is the first one in that location. Instead, count back five sample units and select the sixth one. If the sixth poultry carcass is not a **whole** carcass (**UNTRIMMED**, with or without the neck), then count back five more and take that sixth one. Repeat this procedure until you do select a **whole** carcass.

Note that for swine which are routinely partially skinned, sponge the same locations as those with intact skin.

Now, let's take a step-by-step look at aseptic sampling technique. Because you can't see bacteria when you're sampling, you can't see when and if you accidentally contaminate any items. Contaminated samples could give false results that indicate the process is out of control. Touching, sneezing on or brushing against sampling supplies and the product to sample may contaminate samples. So, always be very conscious of what you and your sampling supplies are touching. Be sure that only sanitized or sterile surfaces contact your sample. This includes your hands and frock.

Except for the sterile peptone water that you refrigerate, keep the sampling supplies you remove from the shipping container in a plastic bag in the government office or other convenient location. This bag will help protect the outer surfaces of the supply packages.

The first step in aseptic sampling is to wash any surface to be sanitized, since sanitizers are only effective when applied to clean surfaces. Sanitize the surface on which you will place your sampling supplies. Sanitize your tote or caddy, if you use one.

After you have prepared your supplies, wash and sanitize your hands up to the mid-forearm. Completely dry them with paper towels. Just before you actually collect the sample, you'll need to put on sterile gloves. Here is the proper way to do it.

Position the glove package so that the letters L and R are facing you. L means left and R means right. When you first open the package, the glove is folded, forming a cuff on the sleeve and lying palm up. Leave the glove in the package until you start to put it on.

Hold the glove open by the inside cuff area. Insert your hand into the glove, palm-side up, and remove the glove from the package. Pull the glove completely on and pull the cuff up without touching the outside surface of the glove with your hand.

Next, place your gloved hand palm up into the cuff of the second glove and put the second glove on. Be careful to avoid touching the inner surface of the glove because you might accidentally touch your skin with the glove you already have on. Even though you washed and sanitized your hands, they are not sterile. **Once both gloves are on, you can touch the outside of a glove with the other gloved hand to adjust the fit.

If at any time you're concerned that a glove may have become contaminated, discard it and repeat this procedure for putting on gloves with another pair of sterile gloves. Remember, you put on your gloves just prior to taking a sample, which we'll show you later.

Now here's a look at sampling. **Every day the product is produced, collect a sample.**

For this you'll need to gather the sampling supplies specific to the product. Be sure all the supplies are readily available before going to the sampling site. Label the sponge bag or sample container before sampling. When the bags or containers are wet or cold, the bar code stickers will not stick.

Wash and sanitize what surfaces your supplies will be contacting. For example, the tote, cart, or table. Prepare the sanitizer as explained in the [*Salmonella Analysis*](#) guidebook.

Wash and sanitize your hands to the mid-forearm and completely dry them with disposable paper towels. The abrasive effect of the towel helps to remove additional contaminants.

We'll review swine, beef, chicken, turkey, and ground product, in that order. The sterile sampling supplies for swine are a pair of gloves, a sponge in a Whirl-Pak bag, a 10 by 10 centimeter template, and 10 milliliters of buffered peptone water. Recall that prior to actually taking the sample, you had randomly determined where and at what time to take the sample, gathered the sampling supplies, labeled the sponge bag, and prepared supply-contact surfaces, as well as your hands.

Now go to the sample location and choose the sample by counting back 5. Allow sufficient room to safely collect your sample beginning with the belly, continuing to the ham and finally, the jowl. By wiping with the sponge in this order of "least to most" contaminated, you'll avoid spreading contamination on the carcass.

Position a ladder or similar equipment near enough to the carcass so you can easily and safely sponge the ham.

Be very conscious to avoid touching sterile surfaces from this point on during the sampling procedure.

Open the sponge bag. Hold it by a corner of the wire closure and tear off the clear perforated strip at the top of the bag. Pull out on the two white tabs to open the mouth of the bag. **Do not touch the inner surface of the bag at any time!** Remove the cap from the buffered peptone water container. Pour all of the buffered peptone water into the sponge bag. Hold the bag closed while you massage the sponge through it. This hastens the sponge's absorption of the buffered peptone water.

When the sponge is fully moistened, carefully push it to the upper part of the bag. Open the bag. The wire closure should keep the bag open, as well as maintain the sponge in place at the opening. Set this aside, but be careful not to contaminate the sponge.

Next, carefully open the template bag, and set it aside and avoid contaminating it.

Put on the sterile gloves as shown earlier. Carefully remove the sponge without touching the bag. Do this with the hand you will use to sponge the carcass. We'll call this your "sampling hand".

With your other hand, remove the template from its bag. Only handle it by the outer edges.

Lay the template over the section of the belly to sample. Notice that this is close to the underarm section. Don't touch the enclosed sampling area or the inner edge of the template with your hands. Use one of two sponging techniques. You may use either of these on the different sites, but always use only one per site. One way is to start at the top of the area in the template. Wipe down firmly but not so hard as to crumble the sponge. Use an even pressure that would be sufficient to remove dried blood. You will need to lift the sponge when you reach the end of one wipe and then rotate it. If you don't lift when you rotate, you may cause the sponge to contact other surfaces. Always keep the same side of the sponge in contact with the carcass. Do this for ten vertical wipes, then turn your hand and do ten horizontal wipes. Each pass of the sponge counts as one wipe. It may be necessary to roll the template while you're sponging since the carcass surface isn't flat.

Next, transfer the template to your "sampling hand". Carefully climb the ladder or platform. Please note your climbing hand is now contaminated. From now on it should not touch the inside of the template, the sample, the sponge or your gloved sampling hand. Use available hand holds or rails with your free hand. Once you're at a convenient and safe height for sampling the ham, transfer the template back to your other hand and lay the template over the ham. Remember not to touch the inner edges of the template or section of the ham you'll sample. Use the same side of the sponge you used for the belly.

For the second sponging technique, wipe downward. When you reach the bottom, lift the sponge and start at the top again. Do this 10 times. Then do the same procedure horizontally. Don't let the sponge contact anything but the area inside the template and, of course, your sampling hand.

Transfer the template again and climb down. Again use caution not to contaminate it with your climbing hand. Turn the sponge over and properly sponge the jowl area with the **unused** side of the sponge. Put the sponge back into its bag. Expel excess air and

fold over the top edge of the bag 3 or 4 times. Then, fold the wire back against the bag. There is no need to double-bag the sample.

Clean up your supplies and store the sample. Later, we'll review storing and shipping the sample.

Let's move on to sampling beef half-carcasses. The procedures for randomly selecting the location and the sample, and then preparing the supplies, sampling area and yourself are the same as for swine. The supplies are also the same.

You'll take the sponging samples at the flank, brisket, and rump, in that order from "least to most" contaminated. For hide-on veal calves, the sample sites are the inside brisket and inside rump. Position a ladder or similar equipment near enough to the carcass so you can easily and safely sponge the rump. Just as was shown for swine, open the sponge bag; moisten the sponge with buffered peptone water; open the template bag; put on the sterile gloves; lay the template over the sample area and sponge 10 times each vertically and horizontally. Roll the template if necessary. Repeat the sponging sequence for the brisket area, using the same side of the sponge.

Carefully transfer the template to your "sampling hand". Safely climb the ladder. Note, your free hand is now contaminated. Do not touch any sterile surfaces with it. Put the template back in your other hand. Turn the sponge over and sample the rump area with the **unused** side of the sponge. Note where on the rump to sample.

Climb down the ladder, again using the handrail. Be careful to avoid contaminating the sponge. Put the sponge back in its bag, then expel excess air and seal it. Clean up your supplies and store the sample.

We'll move on now to sampling chicken carcasses. No sponge is used here because you will rinse the whole carcass in a bag with the buffered peptone water.

Random selection of the site, time and carcass is similar to that of beef and swine. However, here it is important to obtain a **whole** carcass. This means one that has not been trimmed, whether or not it has the neck attached. If the sixth carcass is not whole, count back 5 more and choose *that* sixth one. Note, here he did not chose the fifth carcass, since it was trimmed. Repeat this as often as necessary until you can collect a **whole** carcass.

Take your supplies to the sampling location. These are a pair of sterile gloves, one large and one small zip-lock bag, 400 milliliters of sampling solution, and a sealed container. Prepare the sampling supply contact surfaces and yourself as discussed earlier.

Carefully open the large zip-lock bag. **Do not touch the inside**, which is sterile. Lay the opened bag on its side on a sanitized surface.

Put on sterile gloves as shown earlier. Use only one hand to get the selected carcass by the legs and remove it from the line. For safety, wait for the bird to fall from the shackles before picking it up. Allow excess fluid to drain out.

With your other hand, pick up the open sample bag and place the bird in it so that the vent and legs are toward the bag opening. Remember not to touch the inside of the bag.

Rest the bottom of the bag on a sterile surface, with the bag opened. Uncap the buffered peptone water and pour all of it into the carcass cavity. Expel most of the air from the bag and zip it closed. Support the bottom of the bag with one hand and the top with the other. Invert the bird in the bag 30 times. This takes about one minute. This ensures that all interior and exterior surfaces are rinsed thoroughly.

Set this bag aside and open the small zip-lock bag. Remove the cap from the sample container and place the cap in this bag to prevent it from getting contaminated. **Do not touch the inside of the cap or its container.**

Now, open the bag containing the bird. With one hand, hold the carcass through the bag by the leg. With the other, hold the top corner of the bag to form a "V" at the bottom corner. Use this "V" as a pour spout. Carefully pour the rinse fluid into the open sample container. Fill it only to the 30 milliliter volume line. Set the bag aside again. Take the cap out of the bag and close the sample container of rinse fluid. **Do not touch the inside of the cap.** Make sure the cap is secure.

Put the sample container in the small zip-lock bag, expel excess air, and zip it closed. Pour the remainder of the rinse fluid into a drain. Put the chicken carcass back, either in the chiller or at the location where you'd collected it. Clean up your supplies and store the sample.

Let's move on to turkeys. Turkey carcasses are now sponged by FSIS employees for *Salmonella*, not rinsed as was done previously for the Baseline Survey.

Sponge samples for turkeys are taken similarly to livestock sponge samples. However, the supplies are two pair of sterile gloves, a sponge in a Whirl-Pak bag, 10 milliliters of buffered peptone water, and a 5 by 10 centimeter sterile template. Make sure that the buffered peptone water is not cloudy. Remember to refrigerate the clear buffered peptone water.

You'll prepare your supplies, sampling area and yourself just as was already shown for sampling livestock and chickens. But to prevent the turkey carcass from slipping while you're sampling, you may want to place clean paper towels, tray-pack absorbent pads, or a sanitized wire rack on the sanitized sampling work surface.

Remember to randomly determine the time and location for sampling. Now let's walk through sponging the turkey carcass. There are only two sites on the carcass to sample. These are the back and the thigh.

After you have set up the sampling area, put on one pair of sterile gloves. Use the method shown earlier for putting on gloves without contaminating them. Go to the end of the drip line or chiller and randomly select an intact turkey carcass. Recall that you count back 5. For your safety, wait for the bird to drop off the line before you pick it up. Grasp the turkey by its drumsticks. Do not touch the back or thigh area.

Take the carcass to the sample area and carefully place it breast down on the towels. It's okay if the carcass leans on one side of the breast. But don't let the back or the thigh you are going to sponge touch any supporting surfaces.

Remove and discard the gloves. They are now contaminated from handling the carcass.

Be very conscious to avoid contaminating sterile surfaces from this point on during the sampling procedure.

Just as was shown for swine and beef, open the sponge bag; moisten the sponge with prechilled buffered peptone water; massage the sponge; open the template bag; and put on the sterile gloves.

Carefully remove the sponge without touching the bag. Do this with the hand you will use to sponge the carcass. Remember, we call this your “sampling hand”.

With your other hand, remove the template from its bag. Only handle it by the outer edges.

Lay the template over the section of the back to sample. Notice that this is over the backbone and just in front of the tail. The template should be evenly placed on either side of the backbone. Don't touch the enclosed sampling area or the inner edge of the template with your hands. Use one of the two sponging techniques shown earlier with the swine and beef, making 10 horizontal and 10 vertical wipes. Always keep the same side of the sponge in contact with the carcass. You may need to roll the template.

Next, place the template over one of the thighs. Note that the sample area starts at the hip joint and extends to cover the thigh. Turn the sponge over and use the “clean” side of the sponge, holding it by its edges only and do 10 wipes each vertically and horizontally. Don't let the sponge contact anything but the area inside the template and, of course, your sampling hand.

After sponging the thigh, set the template aside and put the sponge back into its bag. Be careful not to contaminate the sponge or inside of the bag. Expel the air and seal it.

Return the turkey to the point where you collected it; then, clean up your supplies and store the sample.

Our last sample to discuss is ground product. Recall ground product is to be collected after grinding and before final packaging. Preferably you can obtain the sample before any spices or seasonings are added.

Randomly determine the sample location and time. Prepare the sampling area, and if you use one, the tote. Gather and prepare your supplies. These are the sterile gloves, and the plastic wrapped ring template in the Whirl-Pak bag. Wash and sanitize your hands to the mid-forearm. Dry them and begin the process of sampling the ground product.

Open the Whirl-Pak® bag as demonstrated earlier. Push the plastic wrapped ring up to the top of the bag. **Do not touch the plastic wrap or the inner surface of the bag!** Set the bag with its contents aside on a sterile surface. Avoid contaminating them.

Put on the sterile gloves as shown before. Carefully remove the sterile plastic wrapped ring template from the bag, without touching the outside of the bag or any other non-sterile surface. Be sure not to touch or contaminate the inside of the bag.

Open the sterile tape or seal on the plastic wrap and unwrap the ring. Place the sterile sheet on the sanitized work surface. This surface **must** be flat. Put the ring in the center of the sheet.

Without touching anything but the sample and the ring, collect enough raw ground product to fill the ring. Select various portions of the ground meat or poultry to insure that the sample is representative of the batch of product. Set the product you collected on a sterile surface. Firmly pack the sample into the ring to make sure there are no air pockets. Pack the sample level until it is even with the top of the ring. It is critical to fill the ring in this manner. You now have a sample that weighs 25-grams. This is the required sample size for the laboratory analysis. When the lab receives the sample, a lab worker will pour a measured amount of buffer into the bag with the sample. The entire bag, with the product and buffer, is put into a Stomacher to mix it for plating. There must be 25 grams of product in that bag!

Now that you have filled the ring, lift it from the sheet and hold it over the open Whirl-Pak bag. Use your gloved finger and push the ground product out of the ring into the bag. Do not let anything but the sample touch the inside of the bag.

Expel excess air and fold over the top of the bag 3 to 4 times and seal it. Do not double-bag the sample. Clean up your supplies and store the sample. Discard the gloves, plastic sheet and ring.

Well, we've mentioned storing the sample enough times, so let's discuss that now. The sample needs to be maintained at refrigeration temperatures. **DO NOT FREEZE**. Keep it in a **secure** cooler or refrigerator until you pack it into the shipping container. You must ship the sample by overnight delivery on the **same day that you take the sample**. The sample has to be as fresh as possible for analysis.

Finally, we'll take a look at packing the sample for shipment. Retrieve the sample, pre-chilled shipping container and the frozen gel-packs. Place the sample in the shipper. The chicken rinse sample container should be in a zip-lock bag. The sponge sample bags are only sealed by folding the wires over. Do not double-bag the Whirl-Pak bags and do not tape or otherwise seal them. This creates problems at the lab when the lab worker tries to open the bag to pour buffer onto the sponge, which is put into the Stomacher just like the ground sample.

Do not put newspaper or other material in with the sample either to keep the sample upright or to fill the box.

Put a cardboard pad on top of the sample and a gel-pack on top of that. The gel pack keeps the sample cool during shipping. The cardboard prevents the gel-pack from lowering the sample temperature too much and freezing the sample, which could possibly kill bacteria.

Next, place the foam plug on top and press it down as far as it will go. Fill in the "Time Collected" and "Mail/Ship Date" blocks on FSIS Form 10,210-7. Put the form on top of the foam plug. Close and seal the shipping container. Do not tear, or try to remove, old stamp receipts from the shipping container.

Prepare the preaddressed FedEx Stamp. Fill in the plant number, ship date and plant phone number. Sign the stamp and remove the top copy for your records. Arrange for the overnight delivery service to pick up the sample. Apply the stamp over existing FedEx labels. Be sure to cover any barcodes on existing labels with the stamp or a dark marker.

If you are mailing the sample on a Friday, then you will need to attach the special "Saturday Delivery" label to the shipping container. This label has special instructions to the FedEx driver to alert him or her that the lab will accept shipments on Saturdays. Apply this label above the FedEx stamp for Friday shipments only.

If any of the shipping supplies that you need are missing, do not hesitate to contact the Technical Services Laboratory designated on the sample request form to get some.

Remember to maintain sample security at all times.

This concludes the overview of the *Salmonella* analysis sampling procedures. For more information, please consult the guidebook. (documentation, verification, enforcement)

You've just seen in detail how to take a sample for *Salmonella* analysis for the Pathogen Reduction initiative. That act of sampling is by far your most critical and involved role for monitoring *Salmonella*. The example of counting back or ahead five sample units is just that, only an example. You may have some other way of selecting a unit that satisfies random sampling. Also, the types of small buffered peptone water containers that will be supplied by the labs may vary depending upon the supplier. The important issue is that only ten milliliters are used. The sampling methods shown need to be strictly followed to assure uniformity in sample results.

Now let's take a minute to review the performance standards for *Salmonella* that we presented in Session I.

Performance standards are regulatory requirements, enforceable by FSIS. When HACCP is implemented, establishments must consistently meet the pathogen reduction performance standards for *Salmonella* as a condition of maintaining inspection.

The *Salmonella* standards in the regulations are based on a national baseline study conducted by the Agency. FSIS believes all establishments can meet, or do better than, the current baseline prevalence for *Salmonella* contamination by implementing process controls that prevent, and by using food safety technologies and procedures to remove, contamination.

The number of positive test results will be compared to the maximum number of positive results permitted by regulation for that product. *Salmonella* test results only report the presence or absence of the organism, not the number of organisms. Any positive test result indicates *Salmonella* contamination is present.

As I said, the performance standards specify a maximum number of positive test results [*highlight the last column (c)*] permitted in a specified number of samples [*highlight the third column (n)*] for each species and category of raw product [*highlight first column*].

Recall how to use this table from the earlier Session. Let's consider ground beef this time [*highlight line one*]. The performance standard is set at seven point five percent. To meet the standard, an establishment can have no more than five positive sample results out of every fifty-three ground beef samples. Six positives within fifty-three samples signal a failure to meet the standard.

You may have noticed that the numbers in the last two columns do not equal the percentage stated in the second column. The focus of the PR/HACCP Rule is on **process** rather than **product** performance. The series of sample results is only an indicator of process performance, and the results are affected by unavoidable sampling variation. The Agency took this variation into account in setting limits.

The pathogen reduction performance standard applies to establishments, not to individual products. If the establishment fails to meet the standard, it must take corrective actions to lower the incidence of *Salmonella* on all the product of that type it produces. The effectiveness of the corrective action is then measured by subsequent testing. So if in the next fifty-three samples of ground beef the plant had five or less positives, then we would assume that the corrective action was sufficient. But if the standard were again exceeded, we would require the establishment to reassess its HACCP plan and take appropriate corrective actions.

And let's say that on the third consecutive series of fifty-three samples, the establishment again failed to meet the performance standard, then we assume that they cannot maintain sanitary conditions. This then constitutes an SSOP **and** a HACCP systems failure, but the HACCP system is where we focus our actions. FSIS would suspend inspection of the process associated with the HACCP system. For example, if the failed performance standard was for chicken carcasses, then we withhold inspection of the slaughter process, since that's the process that failed. The District Office will provide instructions for further actions.

Let's discuss now what is required of you, the sample collector, when it comes to verification, documentation and enforcement issues after the sample has been analyzed.

As the in-plant inspection personnel required to sample for *Salmonella*, you must collect, process, and mail the sample as directed to determine compliance with the regulatory standard. This language comes directly from the ISP for Procedure code 05A03.

You will not be receiving the sample results, nor will you have to track the results to determine how many samples you've taken.

The **Office of Public Health and Science** will keep the results and the **Sampling Coordination Team** sends them to the District Office on a cycle-by-cycle basis. If the test results indicate that a performance standard was exceeded before the end of the cycle, the **Sampling Coordination Team** will notify the District Office immediately. The District Office will get the *Salmonella* test cycle results. The **Office of Public Health and Science** will make the determination about compliance with the regulatory requirements. When the establishment has failed a pathogen reduction performance standard, the District Office will notify you and give you directions on the course of action. Each failure is handled on a case-by-case basis from the District Office.

So you see, regarding *Salmonella* sampling for the Pathogen Reduction/HACCP Rule, your key role is sampling. If any additional action is needed on your part, the District Office will give you specific instructions at that time.